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An assay for human telomeric G-quadruplex DNA binding drugs

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ABSTRACT

Compounds that stabilize the G-quadruplexes formed by human telomeres can inhibit the telomerase activity and are potential cancer therapies. We have developed an assay for the screening of compounds with high affinity for human telomeric G-quadruplexes (HTG). The assay uses a thiazole orange fluorescent reporter molecule conjugated to the aminoglycoside, neomycin, as a probe in a fluorescence displacement assay. The conjugation of the planar base stacking thiazole orange with the groove binding neomycin results in high affinity probe that can determine the relative binding affinity of high affinity HTG binding drugs in a high throughput format. The robust assay is applicable for the determination of the binding affinity of HTG in the presence of K^+ or Na^+.

Over the last few decades, a number of different telomeric ends have been identified including the human telomeres.[1] The human telomeric ends are non-coding DNA rich in tandem repeats of (TTAGGG). In addition to single and double stranded DNA, the guanine rich telomeres of chromosomal DNA can adopt unique structures formed by the stacking of G-quartets called G-quadruplexes (Fig. 1).[2] Recent work on human telomeres has shown that the human telomeric G-quadruplex (HTG) may be a target for emerging cancer therapies. The interaction of telomeres with the enzyme telomerase has been linked to cancer proliferation.[3–5] However, the formation of G-quadruplexes inhibits the activity of telomerase.[5] Thus, work is under way to find compounds that can stop cancer proliferation by stabilizing G-quadruplexes.

The study of compounds that stabilize HTG is currently lacking in two related areas. First, the number of compounds that bind with high affinity and high specificity is limited. While ligands that interact with different G-quadruplexes have been discovered in the past decade, most of the G-quadruplex ligands contain planar aromatic moieties and stack to G-quadruplexes with moderate binding affinities.[6–11] In order to increase the specificity of compounds for HTG the approach of identifying ligands with high selectivity for G-quadruplex grooves is needed.[6–9]

Aminoglycosides have been shown to bind in the major groove of a variety of nucleic acid targets.[11–16] There is also strong evidence that aminoglycosides bind in the grooves of G-quadruplex DNA.[17] The ability of a drug to bind in the groove of quadruplexes offers the potential for greater selectivity due to the ability to 'sense' differences in groove width as well as interactions with the functional groups in the groove. Therefore, a higher selectivity for G-quadruplex structures can be envisioned by targeting the grooves HTG.

Our approach for increasing the affinity and specificity of HTG binding ligands is the conjugation of aminoglycoside molecules with various planar molecules. When the selectivity of groove binding aminoglycosides is combined with the base stacking surface areas of intercalating molecules with a HTG, ligands with much a higher selectivity[18] and a higher affinity[19] for the G-quadruplex can be designed.

A related limiting factor in the discovery of HTG binding ligands is the lack of a high throughput method of screening molecules that bind in the groove of HTG. In order to develop a screen to determine compound's affinity for HTG, we have synthesized a fluorescent probe by covalently conjugating thiazole orange with neomycin (TO-neo) as shown in Scheme 1. The synthesis of TO-neo (**5**) was achieved by coupling a thiazole orange derivative (**3**) with Boc protected neomycin amine (**4**). The modified thiazole orange derivative (**3**) was synthesized in three steps using a similar procedure reported in literature.[20,21] As displayed in Scheme 1, 3-methyl-2-(methylthio)benzo[d]thiazol-3-ium (**1**) was prepared in one step by reacting 3-methylbenzo[d]thiazole-2(3H)-thione with methyl iodide. In a separate reaction 1-(4-carboxybutyl)-4-methylquinolinium (**2**) was synthesized by reacting 4-methylquinoline with 5-bromovaleric acid. The quaternary salts **1** and **2** were reacted in the presence of triethyl amine to afford compound **3** which bears a carboxylic acid functional group. The Boc protected

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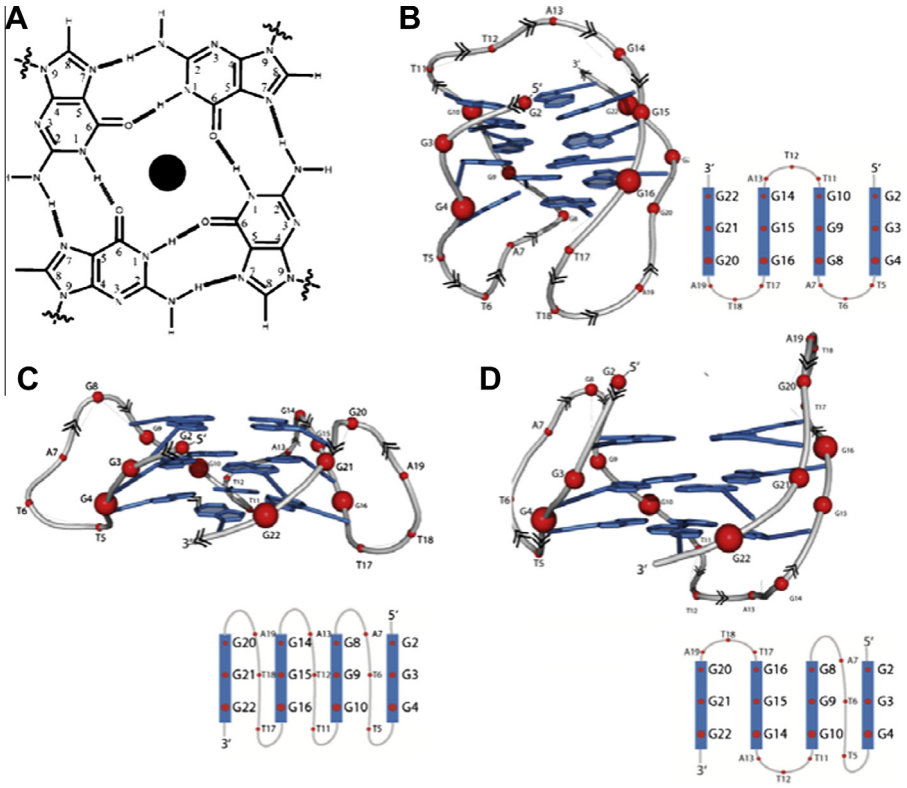
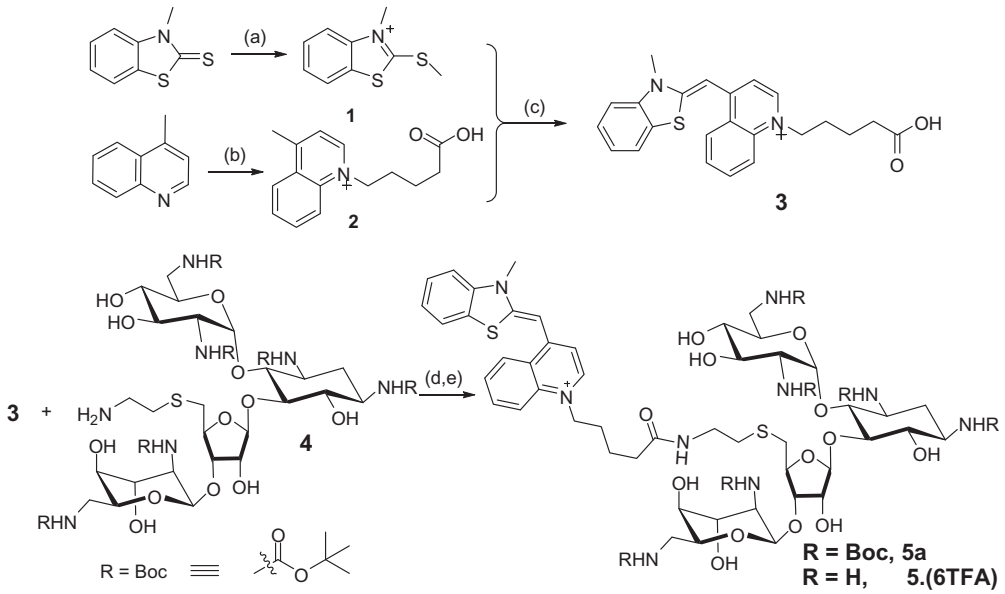


Figure 1. (A) A diagram showing the arrangement of guanines in a G-quartet. Polymorphism of HTG in the presence of sodium and potassium ions (B) a Basket-type conformation in the presence of Na^+ (PDB: 143D) (C) a Propellar-type conformation in the presence of K^+ with parallel strand topology (PDB: 3SC8), and (D) a Hybrid (3 + 1)-type conformation with three parallel and one antiparallel strands (PDB: 2GKU).



Scheme 1. Reagents and conditions: (a) CH_3I, 4 h, 50 °C, 84%; (b) 5-bromovaleric acid, 3 h, 110 °C, 38%; (c) Et_3N, 50 °C for 2 h, then 1 h at room temperature, 24%; (d) DMF, TBTU, DIPEA, room temperature, 65%; (e) TFA, DCM, 3 h, rt, 80%.

neomycin amine (**4**) was synthesized using previously reported procedures.[22,23] The coupling of **3** and **4** was achieved using *O*-(benzotriazol-1-yl)-N,N,N′,N″ tetramethyluronium tetrafluoroborate (TBTU) as the coupling agent to afford Boc protected thiazole orange-neomycin conjugate (**5a**). The Boc protecting groups in **5a** were deprotected using trifluoroacetic acid to afford the desired probe TO-neo (**5**) in 80% yield.

Structural information on HTG has been obtained with solution studies by NMR[2] and in the crystalline state using the sequence d[AGGG(TTAGGG)$_3$].[24] HTG is polymorphic structures and can exist as a parallel strand G-quadruplex[25] or various mixtures of parallel and antiparallel strand G-quadruplex (Fig. 1). The difference in the structure of HTG is highly dependent on the cation present. When formed in 100 mM sodium chloride and 10 mM sodium cacodylate,